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Efficacy of fresh crushed leaves and essential oil of *Eucalyptus camaldulensis* Dehnh on larvae of *Dermestes maculatus* De Geer insect damage to smoked dried *Sardinella aurita* "*Kétiakh*" stored in Senegal

Oumar GUEYE[1*], Néné Gallé FALL[1], El Hadji Barka NDIAYE[2], Khamath SENE[3], Lassana KEITTA[3] and Momar Talla GUEYE[4]

[1]*Atelier Poisson et Produits Halieutiques, Institut de Technologie Alimentaire, Hann- Dakar BP 2765, Sénégal.*
[2]*Département de Chimie, Faculté des Sciences et Techniques, Université Cheikh Anta Diop, BP 5005 Dakar, Sénégal.*
[3]*UFR des Sciences Agronomiques, d'Aquaculture et de Technologie Alimentaire (S2ATA), Université Gaston Berger BP 234 Saint Louis, Sénégal.*
[4]*Laboratoire des Analyses Phytosanitaires, Institut de Technologie Alimentaire, Hann- Dakar BP 2765, Sénégal.*
[*]*Auteur correspondant, E-mail : gueyeniaye87@gmail.com ; omgueye@ita.sn ; Tél.: (+221) 77 427 00 06.*

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ABSTRACT

Synthetic pesticides used to protect stored smoked and dried fish often cause enormous damageto human health and the environment. To limit the harms due to the use of these chemicals, it is necessary to find alternative methods. For this purpose, natural substances may constitute a beneficial way. The objective of this work is to study the effect of crushed fresh leaves and *essential oil* of *Eucalyptus camaldulensis* on the mortality of the larvae of an insect pest of smoked and dried fish, *Sardinella aurita* "*Kétiakh*", *Dermestes maculatus* (De Geer, 1774). Essential Oil was obtained by steam distillation from Eucalyptus leaves (0.8% w/w) and analyses carried out by GC/FID and GC/MS. For the biological test, fresh crushed leaves and essential oil were tested at different doses at varying treatment times again larvae *Dermestes maculates*. The results showed that *D. maculatus* was sensitive for both fresh crushed leaves and the essential oil, the efficacy of which depended on the dose and duration of treatment. The doses of 2 g and 2.5 g of freshly ground leaves showed a high efficiency of 27 to 40%, of mortality respectively. However, for the essential oil, with the increase in the duration of exposure, the highest mortality (33.3%) was recorded after 72 hours of treatment with the 40µl dose and at 120 hours with the 60µl dose. This study showed that fresh crushed leaves and essential oil of *Eucalyptus camaldulensis* can be considered as an alternative to the use of chemical insecticides in the conservation of smoked and dried *Sardinella aurita* "*Kétiakh*"stored in Senegal.

Keywords: *Eucalyptus camaldulensis*, fresh leaves, essential oil, smoked and dried fish "*Kétiakh*", *Sardinella aurita*, *Dermestes maculatus*

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INTRODUCTION

The "*Kétiakh*" (smoked and dried *Sardinella aurita*), is one of the processed fish products that provides an important part of the food for more than half of the Senegalese population. It is also exported throughout West and Central Africa where it is used as food or condiments added to several traditional meals (Fall et al., 2014). Unfortunately, this product is very often characterized by low and unstable yields. This is due, in particular, to their susceptibility to insect infestations (Koch et al., 2016). Indeed, during storage, the "*Kétiakh*" is rapidly attacked by predatory insects such as the Dermestidae beetles, of which *Dermestes maculatus* appears to be the most frequently encountered and in high density on the latter (Gueye-NDiaye, 1991). Storage, which is essential to meet the population's needs for braised, salted and dried "*Kétiakh*" fish and to ensure food security, is a problem to which special attention must be paid (Janin, 2010). Thus, faced with this situation, the processors use chemical products in an abusive way (dosage not respected) making the finished product unfit for consumption (food poisoning, health problems ...). The latter are effective but very dangerous, especially with producers who do not have the necessary knowledge and technicity (Gueye, 2012). The cost, quality, and high toxicity of these products have made it necessary to find alternatives to reduce post-processing losses and increase the availability and safety of traditional fish products for human consumption.

Unfortunately, from their intensive use, many undesirable effects arise such as: environmental pollution, disposal of auxiliaries, food poisoning, bioaccumulation phenomena, etc. (Guèye et al., 2011). To this end, the search for effective but non-chemical alternatives can alleviate, among other things, the problems related to the residues present in the "*Kétiakh*". According to Ndiaye et al. (2018), insects control with repellent products (essential oils or plant-emitted Volatile Organic Compounds, VOCs) seems to be, at least locally, a good alternative to synthetic formulations whose bio-active constituents lead often to insect resistance and toxicity for man and other living organisms. Vegetable substances are among environmentally friendly strategies to reduce health and ecological problems may be a beneficial way forward.

It is in this context of research into alternative control methods to reduce post-processing losses of braised-dried fish, "*Kétiakh*" due to *D. maculatus* as a substitute for synthetic pesticides that this work is being carried out. Essential oils, like those of Eucalyptus, by their larvicidal properties, can be used in the conservation of "*Kétiakh*" as substitutes for pesticides because they are accessible to producers. Indeed, Eucalyptus is planted mainly for its leaves, which have larvicidal effects and medicinal properties but also for their higher content in essential oils. These are exploited for their use in pharmacy and perfumery applications (Elaissi et al., 2012; Siddiqui et al., 2000). Cheng et al. (2009) showed the effectiveness of eucalyptus essential oils against mosquito larvae. Several studies on the chemical composition of essential oils of eucalyptus reported 1.8-cineole as main compound: Francisco et al. (2001) on *E. camaldulensis* of Maputo with 43.4%, *E. camaldulensis* of Morocco 50.69% and in Iran 69.46% and 54.37% (Farah et al., 2002; Medhi et al., 2010; Panahi et al., 2011). The general objective of this study is to test in the laboratory, the effectiveness of essential oil (HE) and fresh crushed leaves of *Eucalyptus camaldulensis* in the protection of stored smoked and dried *Sardinella aurita* against *Dermestes maculatus*.

MATERIALS AND METHODS

Plant material and extraction of essential oil

The fresh leaves of *Eucalyptus camaldulensis* were harvested in Dakar (Hann Zoological Park). Identification of species was confirmed in the Vegetable Biology Department of Cheikh Anta Diop University (Dakar). Botanicals specimens were deposited in the herbarium of "Institut Fondamental d'Afrique Noire" (IFAN) of Cheikh Anta Diop University (Dakar).

The freshly harvested plant material was washed with distilled water. One part was dried for 3 days in the shade at room

temperature and the other part is freshly crushed.

To extract the essential oil, the *Eucalyptus camaldulensis* dried for 3 days are subjected to steam training for 4 hours (with 1.5 liter of water) using a Clevenger-type device.

Analysis of essential oils

Oils and extracts from aqueous distillates were analysed by gas chromatography fitted with a flame ionization detector (GC-FID) and gas chromatography coupled with a mass spectrometer (GC-MS).

GC-FID: The gas chromatograph fitted with a flame ionization detector (Thermo-Trace, Interscience, Belgium) was equipped with an optima-5-MS Accent capillary column from Agilent (Belgium) (30 m x 0.25 mm x 050 µm film thickness for a complete resolution of 1,8-cineole and limonene which co-eluted with thinner stationary phases). The oven temperature ranges from 40 to 250 °C according to the following programme: 40 °C for 3 min and then a programmation at 5 °C/min until 250 °C with a final hold of 5 min at this temperature. Helium (He) was used as carrier gas at a flow rate of 1.1 ml/min. The injector used in splitless mode was set at 280 °C. The detector (FID) temperature was 280 °C. The FID runs with compressed air (at a flow of 350 mL/min) and hydrogen (35 mL/min). A make-up gas (N2) was used with a flow rate of 30 mL/min. GC-MS: The gas chromatograph (Agilent 6890-USA) equipped with MS (Agilent 5973 NETWORK mass selective detector) in the electron impact mode (70 eV) source and quadrupole temperatures were of 280 and 150 °C, respectively. The scanned mass range was fixed at 35–350 amu. The column was the same than GC-FID. The oven temperature was programmed as follows: isotherm of 5 min at 40 °C then a progression of 8 °C/min up to 280 °C with a final hold of 5 min at 280 °C. The injector, used in splitless mode, was at 240 °C. 1 µl of each sample was injected. The carrier gas was Helium (He) with a constant flow rate of 1.1 mL/min. The identification of the compounds was made using data of computer library (Wiley 275L) connected to the GC-MS and retention indices of components were calculated using retention times of n-alkanes (C7-C30) and compared with those of the literature (Joulain and Konig, 1998 ; Adams, 2001).

Insects used

D. maculatus adults used were collected at Mbour specifically at the Mballing processing site from infested dried braised fish. Then, they were maintained in mass breeding under the laboratory environmental conditions (temperature (22 ± 4 °C) and relative humidity (80 ± 5%)) in one liter pots. After emergence, larvae from mass rearing were used for toxicity testing.

Dried and smoked fish *Sardinella aurita* "*Kétiakh*"

Dried and smoked *Sardinella aurita* samples used for the mass rearing of adults of *D. maculatus* and the others bioassay tests were bought on a Dakar market (Castor). They are not subjected to any treatment with insecticides or other chemical compounds that may influence testing.

Toxicity testing

Fumigation test with *Eucalyptus camaldulensis* essential oil

Five larvae of *D. maculatus* were placed with 20 g of dried and smoked *Sardinella aurita* in half-liter jars. Doses of 60 µL, 70 µL, 80 µL and 90 µL of *Eucalyptus camaldulensis* essential oil were tested (Table 1). The essential oil was deposited using a micropipette on a washer of filter paper stuck to the wall of the lid of each jar for good diffusion and also to avoid direct contact with the product. Each treatment was repeated 3 times.

Contact test with fresh crushed leaves of *Eucalyptus camaldulensis*

The experimental design was subdivided into 3 treatments (Table 1) repeated three times. The tests were carried out in glass jars of ½ liter with perforated lids to allow good ventilation. The method is to introduce in each jar, 20 g of dried and smoked *Sardinella aurita* with the doses 2 g, 2.5 g and 3 g of crushed fresh leaves. After the introduction of the

different doses, the jars were heavily shaken (1 to 2 min) and then stabilized for 5 to 10 min in order to homogenize the deposit of particles and then five larvae of *D. maculatus* were introduced into each jar. An untreated control and a control treated with Actellic (1.6% pirimiphos-methyl and 0.3% permethrin by mass) were used as references for the toxicity tests.

Mortality evaluation

Daily insect mortality monitoring was carried out over 24, 72 and 120 hours. The evaluation consisted of the census of dead insects and was removed from the jars while the survivors were returned to the jars. Abbott, (1925) formula below was used to correct for natural mortality.

$$\text{Mcorr} = (\frac{\text{Mt} - \text{Mc}}{100 - \text{Mc}})\text{x}100$$

Mcorr = adjusted mortality (%)
Mt = Mortality in the treated group
Mc = Mortality in the negative control group

Statistical analysis

Statistical analyses of the variables measured were performed with XL-STAT 6.1.9 software. The data obtained were subjected to the ANOVA variance analysis and the averages compared by the Fisher test at 5%.

Table 1 : Experimental design.

Formulation	Control		*Eucalyptus camaldulensis* essential oil			Fresh crushed leaves of *Eucalyptus camaldulensis*		
	Négative	Positive (actellic)	Dose (µL)			Fresh leaves (g)		
Treatment	T0	T1	T2	T3	T4	T5	T6	T7
Dose	0	0,125	20	40	60	2	2,5	3

RESULTS

Gas chromatographic analysis

The results of essential oils analysis are presented in Table 2. A total of 57 compounds were identified in the volatile profile of the essential oils from Dakar, representing more than 94.5% of the total oil components which were detected (Table 1). The main constituent was β-pinene at 26.8%, α-eudesmol (19.2%), epiglobulol (7.9) γ-eudesmol (6.1%) and α-pinene (4.5%). These percentages of β-pinene and α-pinene explain the high content of hydrocarbon monoterpenes with 41.0%. This chemical group was marked by the presence of α-thujene (0.2), myrcene (0.5%), limonène (4.4%) and β-phellandrene (2.0%). Oxygenated sesquiterpenes were present with (38.8%). Hydrocarbon sesquiterpenes such as γ-gurjunene (1.1%) bicyclogermacrène (1.1%) aromadendrène (0.8%), β-caryophyllene (0.7%) γ-cadinene (0.6%) and alloaromadendréne (0.5%) were present at 6.9%. However, there was a low content of 1.8-cineole (2.6%).

Effect of the *Eucalyptus camaldulensis* essential oil on the larvae of *Dermestes maculatus*

Figure 1 shows the efficacy of *Eucalyptus camaldulensis* essential oil against *Dermestes maculatus* larvae. No mortality was observed in the untreated control, in contrast to the control treated with actellic where no insect survived. With exposure duration of 24 hours, the *Eucalyptus camaldulensis* essential oil caused low mortalities which slightly exceeded 10% at all applied doses. However, with increasing duration of exposure, the highest

mortality (33.3%) was recorded after 72 hours of treatment at a dose of 40 µl and at 120 hours at a dose of 60 µl.

Effect of crushed fresh leaves of *Eucalyptus camaldulensis* on larvae of *Dermestes maculatus*

Figure 2 shows the efficiency of fresh crushed leaves of *Eucalyptus camaldulensis* on the mortality of *Dermestes maculatus* larvae. There is no mortality with the untreated control and full mortality with the actellic-treated control. Both the 24 hours and 72 hours exposures showed very low mortality rates, less than 15% at all dose. However, with the 120 hours duration, the 40 µl dose is more effective than the 20 µl dose on *D. maculatus* mortality with maxima of 40% for T6 and 26% for T5.

Table 2: Chemical constituents of essential oil from *Eucalyptus camadulensis* leaves collected from Dakar.

Compounds	RIa	RI b	Area (%)
α-thujene	924	928	0.2 ± **0.1**
α-pinene	932	936	4.5 ± **1.0**
camphene	936	953	0.1 ± **0.0**
β-pinene	974	982	26.8 ± **0.6**
myrcene	988	988	0.5 ± **0.1**
α-phellandrene	1002	1008	0.1 ± **0.0**
α-terpinene	1014	1019	tr
p-cymene	1022	1027	2.2 ± **0.2**
Limonene	1024	1032	4.4 ± **0.4**
β-phellandrene	1026	1033	2.0 ± **0.4**
1.8-cineole	1026	1036	2.6 ± **0.2**
γ-terpinene	1054	1060	0.1 ± **0.0**
α-terpinolene	1086	1088	0.1 ± **0.1**
Linalool	1097	1103	0.1 ± **0.0**
fenchol « exo »	1122	1125	0.2 ± **0.0**
α-campholenal	1126	1128	0.1 ±**0.0**
trans-pinocarveol	1139	1145	0.6 ±**0.1**
pinocarvone	1165	1169	0.3 ± **0.1**
borneol	1169	1183	0.1 ± **0.0**
terpinene-4ol	1177	1186	0.8 ± **0.1**
P-cymen-8ol	1183	1189	0.1 ± **0.0**
α-terpineol	1189	1193	0.4 ± **0.1**
trans-p-mentha-1-(7)-8-dien-2ol	-	1199	1.0 ± **0.1**
myrtenol	1196	1202	1.0 ± **0.1**
trans-carveol	1217	1222	0.1 ± **0.0**
phellandral	1273	1285	0.2 ± **0.0**

thymol	1290	1292	0.1 ± **0.0**
carvacrol	1299	1297	0.1 ± **0.0**
bicycloelemene	1333	1339	0.6 ± **0.4**
α-copaene	1377	1380	0.1 ± **0.0**
α-elemene	1381	1385	0.3 ± **0.0**
β-elemène	1391	1396	0.3 ± **0.0**
α-gurgunene	1410	1419	0.1 ± **0.0**
β-caryophyllene	1421	1433	0.7 ± **0.1**
β-gurjunene	1434	1440	0.1 ± **0.0**
calarene	1437	1448	0.1 ± **0.0**
Aromadendrene	1441	1452	0.8 ± **0.1**
α-humulene	1454	1458	0.1 ± **0.0**
alloaromadendrène	1460	1469	0.5 ± **0.0**
γ-gurjunene	1477	1474	1.1 ± **0.0**
germacrène D	1485	1487	0.1 ± **0.0**
ledene	1491	1496	0.1 ± **0.0**
bicyclogermacrene	1500	1503	1.1 ± **0.0**
γ-cadinene	1514	1508	0.6 ± **0.2**
δ-cadinene	1523	1523	0.1 ± **0.0**
cis-calamenene	-	1526	0.1 ± **0.0**
elemol	1550	1556	1.4 ± **0.1**
spathunelol	1578	1576	0.1 ± **0.0**
globulol	1585	1585	0.4 ± **0.1**
epiglobulol	-	1591	7.9 ± **0.1**
viridiflorol	1593	1598	2.0 ± **0.2**
10-epiγ-eudesmol	1619	1621	1.0 ± **0.1**
γ-eudesmol	1632	1645	6.1 ± **0.7**
isospathulenol	1637	1649	0.2 ± **0.0**
hinesol	1642	1653	0.4 ± **0.0**
β-eudesmol	1651	1660	0.1 ± **0.0**
α-eudesmol	1654	1668	19.2 ± **0.7**
hydrocarbon monoterpenes			
Oxygenated Monoterpenes		**41.0**	
Hydrocarbon sesquiterpenes		**7.8**	
Oxygenated sesquiterpenes		**6.9**	
Total identified (%)		**38.8**	
		94.5	

RIa = Retention index (Adams 2001 or Joulain and könig 1998) **RIb** = Retention index to C7-C30

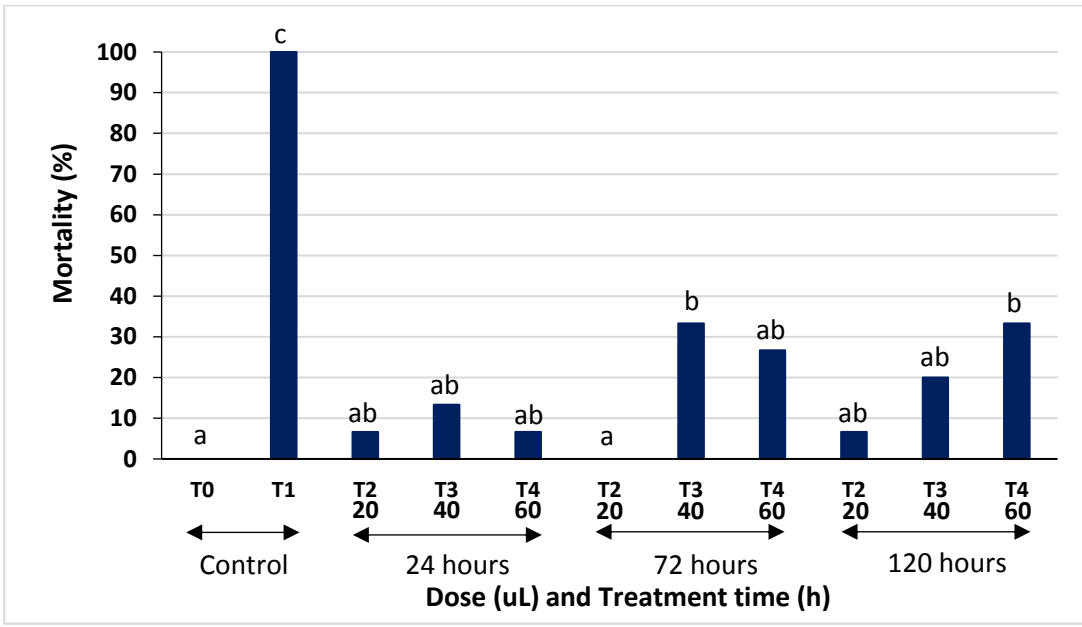

Figure 1: Effect of *Eucalyptus camaldulensis* essential oil on the mortality of *Dermestes maculatus* larvae as a function of dose and treatment time. Histograms followed by the same letter(s) are not statistically different ($\alpha \leq 0.05$).

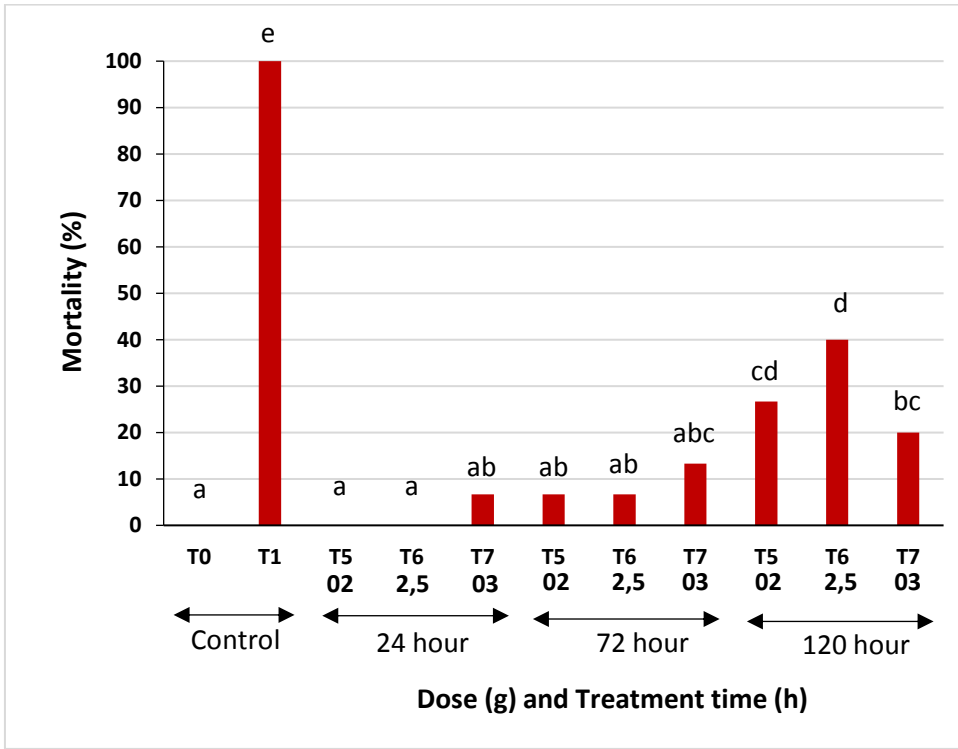

Figure 2: Effect of crushed fresh leaves of *Eucalyptus camaldulensis* on mortality of *Dermestes maculatus* larvae as a function of mass and treatment time. The histograms followed by the same letter are not statistically different ($\alpha \leq 0.05$).

DISCUSSION

The chemical composition of essential oils revealed high percentage of hydrocarbon monoterpenes (β-pinène, α-pinene and limonene). The major compounds were α-pinene (22.5%) and α-phellandrene (20.1%) as majority compounds and hydrocarbon monoterpenes as majority group (Cheng et al., 2009). These chemical features are close to those reported from the same species in Taiwan.

Oils from both of our samples contain low percentage of 1.8-cineole (2.55 %). This compound has often been reported as a major component in essential oil from Eucalyptus. Francisco et al. (2001) found on *E. camaldulensis* collected from Maputo 43.4% of 1.8-cineole, *E. camaldulensis* of Morocco revelead 50.69% and those of Iran 54.37% (Farah et al., 2002; Panahi et al., 2011). Though low in 1.8-cineole, percentages of our oils exceed those of an *E. camaldulensis*'s oil of Algeria (Elaissi et al., 2012). The difference in composition of our oil and that of the other studies can be due to several factors. Thus, Figueiredo et al. (2010) reported abiotic (geographic variation, climat pollution…) and biotic (genetic factors and evolution, diseases and pests…) factors can impact the essential oils compositions.

The results of biological tests show that essential oil and the fresh crushed leaves of *Eucalyptus camaldulensis* can have a larvicidal effect. Indeed, the treatments act on the mortality of *D. maculatus* larvae, however these effects are strongly related to the dose used and the time of treatment.

The results of treatments with *Eucalyptus camaldulensis* essential oil reveal overall that *D. maculatus* is sensitive to tests especially with the 60 µL dose for duration of exposure of 120 hours where mortality is 33.3%. The mortality of *D. maculatus* larvae observed on dried and smoked *Sardinella aurita* treated with the essential oil could be due to a toxic effect of these compounds like β-pinene and α-pinene. Indeed, Kounnki et al. (2007) reported that hydrocarbon monoterpenes like α-pinene, β-pinene and Δ-3-careen are toxic against insects and act as synergy. Lee et al. (2004) show the efficacy of eucalyptus essential oil against three types of insect pests like *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica*.

Tests with fresh crushed leaves of *Eucalyptus camaldulensis* gave more satisfactory results compared to treatments with the essential oil. Indeed, the mortality rate obtained is very low after 72 hours of exposure but reached 40% (p-value < 0.0001) after 120 hours with the 2.5 g dose. This larval mortality can also be due to the main active compounds present in the leaves of *Eucalyptus camaldulensis*. The insecticidal property of any plant material would depend on these active constituents. Indeed, the use of plant extracts (essential oils, plant powder, aqueous extracts...) for the control of insect pests of stored products has been widely reported (Fasakin, 2003; Mbaye et al., 2012; Jose et al., 2014). Mbaye et al. (2012) were obtained a repellent and biocidal activity of *Crataeva Religiosa* leaf powder against various stages of *Dermestes spp*. Similarly, Fasakin (2003) have demonstrated a potent effect of oil extracts from *Piper guineense*, *Monodora myristica*, *Aframomum melegueta*, *Tithonia diversifolia* and *Nicotiana tabacum* for killing all adults, pupae and eggs of *D. maculatus*, insect pest of smoked fish. According to Jose et al. (2014), *Secamone afzelii* powder at a dose of 3 g caused 48.8% mortality of *Dermestes maculatus* larvae in 72 hours and 56.8% mortality in 120 hours of exposure to the powder. These results are in accordance with ours.

The results are significant but the mortality rates obtained are low. This could be due probably to the volatility, low doses used and also to the duration of exposure of the essential oil from *E. camaldulensis* fresh leaves.

Conclusion

The present study showed that fresh crushed leaves and the essential oil of *Eucalyptus camaldulensis* have interesting insecticidal properties against *Dermestes maculatus* larvaes. The experiments conducted in this study suggest the possibility of their use in protecting dried and smoked *Sardinella aurita* stocks; indeed low doses cause

significant mortality rates. Crushed fresh leaves and the essential oil of *Eucalyptus camaldulensis* are a promising alternative to synthetic pesticides widely used and which are the cause of many public health problems. The larvicidal properties of the essential oil and fresh leaves of *Eucalyptus camaldulensis* are therefore useful in improving the sanitary quality and extending the shelf life of dried braised fish. A broader study on the others pests of stored dried and smoked *Sardinella aurita* would circumscribe its spectrum of effectiveness.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

OG contributed to writing this manuscript and performed all the data analyses. NGF and MTG contributed to revising the manuscript. KS and LK contributed to the study design. EBN performed all the analyses of the essential oil.

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